

--The PCR product which will be the wild-type adenylate kinase gene or alternatively a mutant adenylate kinase gene already known to produce thermolabile adenylate kinase such as strain CV2 is then cloned into a suitable vector, such as the pALTER-1 from Promega, a plasmid based on pBR322. This has disabled antibiotic resistance genes to facilitate mutagenesis. [CV2 is known (Proc. Natl. Acad. Sci. USA, (1970) 65:737) and may be obtained from the *E. coli* Genetic Stock Centre, 355 Osborn Memorial Laboratories, Box 208104, Yale University, New Haven, CT 06520-8104, USA.]--

#### **IN THE CLAIMS**

Amend the claims as follows.

Cancel claims 19-32, without prejudice.

Add the following claims:

--33. (new) A method for producing a thermostable luciferase which is substantially free of adenylate kinase, the method comprising culturing a host cell which is able to express said thermostable luciferase and which is able to express adenylate kinase only in a mutant form which form has adenylate kinase activity under culture conditions but loses said activity under conditions of pH or temperature at which the thermostable luciferase remains unaffected; and recovering the thermostable luciferase, wherein either the host cell culture or the recovered thermostable luciferase is subjected for a sufficient period of time to conditions of pH or temperature under which the adenylate kinase is denatured but the luciferase remains unaffected and wherein where

the thermostable luciferase is subjected to temperature conditions, the temperature is elevated to 37°C or more.

34. (new) A method according to claim 33 wherein the host cells are cultures for a period which is sufficient to allow production of luciferase, and then a batch of said culture is subjected to said conditions of pH or temperature under which the adenylate kinase is denatured, and the luciferase is recovered.

35. (new) A method according to claim 33 wherein the conditions at which the adenylate kinase is denatured and the luciferase remains unaffected are temperature conditions.

36. (new) A method according to claim 33 wherein said conditions at which the adenylate kinase is denatured and the luciferase remains unaffected are pH conditions.

37. (new) A method according to claim 33 wherein the adenylate kinase includes mutations at amino acids 87 or 107 in the sequence of *E. coli* adenylate kinase.

38. (new) A recombinant cell which comprises a first nucleotide sequence which encodes a luciferase protein under the control of regulatory elements which allow expression of said luciferase protein, and wherein a gene which encodes adenylate kinase is mutated such that the adenylate kinase expressed is denatured under pH or temperature conditions of 37°C or more at which the enzymatic activity of the luciferase is not adversely affected.

39. (new) A recombinant cell according to claim 38 which further comprises at least one selection marker.

40. (new) A recombinant cell according to claim 38 which is a prokaryotic cell.

41. (new) A recombinant cell according to claim 40 which comprises a recombinant *E. coli* cell.

42. (new) A method for producing a recombinant cell according to claim 38 which method comprises in any order (a) transforming a host cell with a vector which encodes said adenylate kinase in a form which is denatured under conditions of pH or temperature at which the enzymatic activity of the luciferase protein is not adversely affected, subjecting transformants to said conditions and detecting those transformants in which adenylate kinase is denatured, and (b) transforming said host cell with a vector which encodes a luciferase which retains a luciferase activity under said conditions and a first selection marker, and detecting transformation with said first selection marker.

43. (new) A method according to claim 42 wherein the vector which encodes a said adenylate kinase in a form which is denatured under conditions of pH or temperature at which the enzymatic activity of the luciferase protein is not adversely affected further comprises a second selection marker which is said first selection marker, and selecting said transformants with said second selection marker.

44. (new) A method according to claim 43 wherein said first selection marker and said second selection marker comprise particular different antibiotic resistance genes.

45. (new) A method according to claim 43 wherein said second selection marker and said second selection marker comprise particular different antibiotic resistance genes.

46. (new) A method according to claim 33 wherein the adenylate kinase includes